onds. 125 μl of the binding protein reagent is added and incubated an additional 215 seconds. 100 μl of solution **1** is added and incubated an additional 375 seconds. 100 μl of solution **2** is added and incubated an additional 590 seconds. About 20 μl is then removed from the reaction mixture and placed in an electrochemical cell and a measurement made as described above. The current measured is related to the sample B_{12} concentration through a standard curve.

EXAMPLE 7

Folate Assay

The folate CEDIA® assay, commercially available from Boehringer Mannheim Corporation, may be modified as described below in order to perform an assay for folate according to the present invention. The modification to the folate CEDIA® assay would be carried out in a similar manner to the Theophylline System Pack modification described above in Example 1.

The ED reagent, which contains ED bound to pteroylglutamic acid, is modified by using an electrochemically labeled substrate in place of the colorimetically labeled substrate provided in the commercial kit. The EA reagent, ED and EA buffers, pre-treatment reagent, and folate binding protein are unmodified from the commercial kit.

A folate assay may be carried out as follows. ED reagent is reconstituted with 15 ml ED buffer to form solution **1**. EA reagent is reconstituted with 15 ml EA buffer to form solution **2**. The folate binding protein is reconstituted with 15 ml ED buffer to form a binding protein solution. 42 μl of a sample containing folate is added to 42 μl of pre-treatment reagent and incubated 80 seconds. 100 μl of the binding protein solution is added and incubated an additional 215 seconds. 100 μl of solution **1** is added and incubated an additional 395 seconds. 100 μl of solution **2** is added and incubated an additional 590 seconds. About 20 μl is then removed from the reaction mixture and placed in an electrochemical cell and a measurement made as described above. The current measured is related to the sample B_{12} concentration through a standard curve.

The present invention has been disclosed in the above teachings and drawings with sufficient clarity and conciseness to enable one skilled in the art to make and use the invention, to know the best mode for carrying out the invention, and to distinguish it from other inventions and from what is old. Many variations and obvious adaptations of the invention will readily come to mind, and these are intended to be contained within the scope of the invention as claimed below.

What is claimed is:

1. A diagnostic kit for determining the presence or concentration of an analyte in a fluid sample, comprising:

(a) an enzyme donor reagent which comprises
  1) an enzyme donor polypeptide conjugate; and
  2) a labeled substrate, comprising an enzyme substrate cleavably linked to an electroactive label;

(b) an enzyme acceptor reagent which comprises
  1) an enzyme acceptor polypeptide capable of combining with the enzyme donor polypeptide conjugate to form an active enzyme complex capable of catalyzing the cleavage of the electroactive label from the enzyme substrate; and
  2) a first antibody capable of immunologically, competitively binding to the analyte and the enzyme donor polypeptide conjugate and hindering formation of the active enzyme complex when bound to the enzyme donor polypeptide conjugate; and

(c) an electrochemical immunosensor.

2. The diagnostic kit of claim **1**, wherein the enzyme donor polypeptide conjugate is a conjugate of an enzyme donor polypeptide and at least one of an analyte or an analyte analog.

3. The diagnostic kit of claim **2**,wherein the enzyme acceptor reagent further comprises an amount of the analyte sufficient to ensure that changes in concentration of the analyte in the fluid sample are substantially linearly related to changes in the current measured by an electrochemical measurement.

4. The diagnostic kit of claim **3**, wherein the enzyme donor reagent further comprises an amount of added peptides, protein fragments, or proteins sufficient to reduce hydrolysis of the enzyme donor polypeptide conjugate from proteases in the fluid sample.

5. The diagnostic kit of claim **2**, wherein the enzyme acceptor reagent further comprises a second antibody capable of immunologically binding to the first antibody, thereby further hindering formation of the active enzyme complex when the first antibody is bound to the enzyme donor polypeptide conjugate.

6. The diagnostic kit of claim **1**, wherein the electrochemical immunosensor includes a first insulating substrate, first and second electrodes affixed to the first insulating substrate, and a second insulating substrate, which overlays the first and second electrodes has a window for exposing at least a portion of the first and second electrodes, and has a cutout portion at one end to allow contact between the electrodes and a meter and a power source.

7. The diagnostic kit of claim **6**, wherein the first electrode is a working electrode and the second electrode is a counter electrode.

8. The diagnostic kit of claim **7**, wherein the working and counter electrodes are palladium, platinum, gold, silver, titanium, copper, or carbon.

9. The diagnostic kit of claim **6**, wherein the first electrode is a working electrode and the second electrode is a reference electrode.

10. The diagnostic kit of claim **1**, wherein the enzyme substrate comprises μ-D-galactopyranoside and the electroactive label comprises 4-(1,4,7,10-tetraoxadecyl)-1-naphthyl, 4-methoxy-1-naphthyl, p-aminophenyl, p-nitrophenyl, chlorophenol red, o-nitrophenyl, umbelliferyl, o-methoxy-p-nitrophenyl, 3,4 dinitrophenyl, m-cyano-p-nitrophenyl, 4-nitrosalicylaldehyde, or 4-methyl-umelliferyl.

11. An electrochemical immunoassay method for determining the presence or concentration of an analyte in a fluid sample, comprising:

(a) preparing a mixture which includes;
  (1) the fluid sample;
  (2) an enzyme donor reagent, which comprises:
    (i) an enzyme donor polypeptide conjugate; and
    (ii) a labeled substrate, comprising an enzyme substrate cleavably linked to an electroactive label; and
  (3) an enzyme acceptor reagent, which comprises (i) an enzyme acceptor polypeptide capable of combining with the enzyme donor polypeptide conjugate to form an active enzyme complex capable of catalyzing the cleavage of the electroactive label from the enzyme substrate; and (ii) a first antibody capable of immunologically, competitively binding to the analyte and the enzyme donor polypeptide conjugate and hindering formation of the active enzyme complex when bound to the enzyme donor polypeptide conjugate;